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Applied Innovative Research
Vol. 2, March 2020, pp. 39-43

Biosynthesis of silver nanoparticles from flowers of *Rhododenderon campanulatum* tree of Tungnath Himalaya

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Recieved 31 January 2020

Rhododenderon campanulatum is a decumbent shrub of *Ericaceae* family. In India, it is distributed (in alpine region from 3000m-3640m *asl*) in Jammu and Kashmir, Uttarakhand and Himachal Pradesh. It is commonly known as white burans. Phytochemical screening of methanolic and aqueous extract of *R. Campanulatum* reveals the presence of glycosides, flavonoids, tannin, saponin in, etc. It is toxic in nature. The present study deals with the green synthesis of silver nanoparticles. In green synthesis of Ag-NPs, the silver nitrate solution and the aqueous plant extract were mixed well in the different ratios.The metal ion present in the solution reduced as Ag^{+1} to Ag^{0} within three days. In the spectrophotometric observation the λ_{max} peak of Ag-NPs of *R. campanulatum* flowers (RCF) obtained at 464nm for 1mM($RCF_{1:9}$), 492nm for 3mM($RCF_{1:9}$), 472nm for 5mM($RCF_{1:11}$). In the SEM analysis the SEM images reveled that the average grain size of $5RCF_{1:11}$AgNPs is ranging from 3µm - 4µm with spherical clusters, while in the EDAX analysis Ag (69.14 weight %), C(8.62 weight %) and O(22.24 weight%) elements are present in the synthesized silver nanoparticles of 5mM($RCF_{1:11}$). Which are further characterized by XRD, TEM etc.

Keywords: *Rhododendron*, Silver nanaoparticles, Biosynthesis

1 Introduction

The genus *Rhododendron* belongs to the heather family, *Ericaceae*. *Rhododendron* is a Northern Hemisphere genus, distributed from North America across Europe, Asia to Japan and in India (Kashmir, Himachal, Uttarakhand, Valley of flower, Tungnath, Adhwani, Binser and Bharsar region). The first record of a *Rhododendron* in cultivation in Britain is of *R. hirsutum*in 1650. This is also a native of Portugal and southern Spain. *Rhododendron campanulatum* discovered from Nepal in 1825[1]. *Rhododenderon campanulatum* is a decumbent shrub of white colour flowers, so it is known as white burans(picture shown in Fig. 1), Simris, Shargal, Semru in Uttarakhand. The plant has a height upto 6m long and oval shaped leaves at an altitude of 3000-4200 meters.

Plant Name : *Rhododendron campanulatum*	Family : *Ericaceae*
Species : *R.campanulatum*	Order : Ericales
Local name : White Burans, Shargal	Habitat : Alpine shrubberies
Taxonomy : Ovate leaves, red spotted stems, white pink spotted flowers.	

This plant can tolerate harsh climatic conditions. The flowers and leaves of *R. campanulatum*are toxic in nature but when mixed with tobacco useful in cold, chronic rheumatism, syphilis, sciatica and hemicranias while bark is medicinal for digestive and respiratory disorders while roots of *R. campanulatum* used as medicine to alleviate many disease like boils, cough, headache, tonic, fever, etc.[2] The red patches are present on the stem of the *R. campanulatum*, these twigs are used in Nepal as medicine in phthisis and chronic fever[3]. It is useful for fuel and charcoal. The tree of white burans has so many medicinal properties and potent antimicrobial activity which may be helpful ingenerating lead molecules for the development of new and novel antibacterial agents by nanoparticle synthesis[4].

Nanoparticles can be defined as sub nano sized colloidal structures with particles size between 1 and 100nm [5]. The silver nanoparticles have large number of applications: nonlinear optics, spectrally selective coating for solar energy absorption, biolabeling, electrical batteries, catalyst in chemical reactions, antibacterial materials, chemically stable materials and good electrical conductors[6-7]. Research in nanotechnology especially for green chemistry pathways to fabricate technologically important nanomaterials is an immense place of interest[8]. Green synthesis of nanoparticle is an ecofriendly technology

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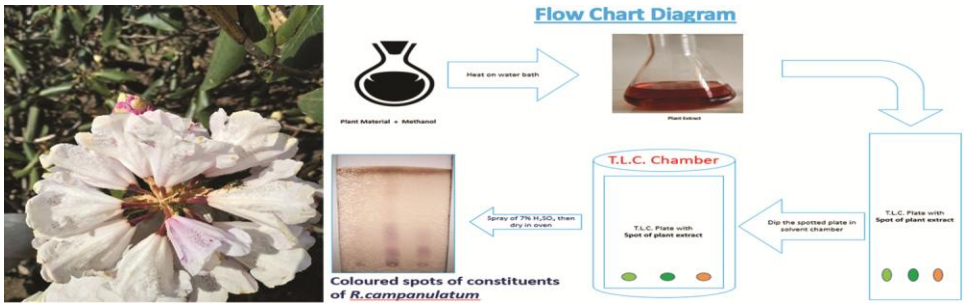


Fig. 1 — *R. campanulatum* flowers (RCF) and flow chart diagram for TLC process (Capture and created by author).

to material chemistry field which includes the synthesis of nanoparticle by bio reduction reaction. The need in this hour to find new source for the synthesis of nanoparticle because the metallic nanoparticles can be fabricated by biological source[9,10].

2 Materials and Methods

The leaves and flowers of *R. campanulatum* (white burans) was collected from Tungnath Himalaya region of Garhwal, Uttarakhand and identified by Taxanomist, Department of Botany HNB Garhwal University Srinagar Garhwal, where the voucher specimens (GUH 0743) was deposited[11].

For the preliminary investigation of phyto-constituents plant material was extracted with methanol as solvent in a round bottom flask on heating mantle at nearly 60-70°C for 2 h. The extract was prepared and cooled at room temperature and chromatographic studies were made. Thin layer chromatographic studies revealed that the resulting mixture has a range of phyto-constituents. On development of TLC plate in solvent ($CHCl_3$: MeOH: H_2O, 32 System, mobile phase) with the help of TLC and as developing solvent take 7% H_2SO_4 shows the presence of several spots based on their polarity (as shown in Fig. 1).

3 Green Synthesis of Silver Nanoparticles

In the green synthesis of silver nanoparticles from leaves and flowers of *R. campanulatum*, dried in shade for 15-30 days, crushed the well dried leaves and flowers of *R. campanulatum.*

Take 5gm of well dried leaves in 100ml deionised water in a round bottom flask. Heat the round bottom flask on heating mantle at 70-80°C for 30 min. Prepared leaves extract filtered two times on whattman filter paper no.1, then mixed with silver nitrate ($AgNO_3$) solution of different concentration, viz. 1mM, 2mM, 3mM in following ratios (Table 1).

Table 1

S. NO.	Concentration of $AgNO_3$ (in mM)	Leaves Extract: $AgNO_3$
1.	1 milli Molar	1:9, 1:10, 1:11
2.	2 milli Molar	1:9, 1:10, 1:11
3.	3 milli Molar	1:9, 1:10, 1:11

Table 2

S. NO.	Concentration of $AgNO_3$ (in mM)	Flowers Extract: $AgNO_3$
1.	1 milli Molar	1:9, 1:10, 1:11
2.	3 milli Molar	1:9, 1:10, 1:11
3.	5 milli Molar	1:9, 1:10, 1:11

Similarly prepare the flowers extract of *R. campanulatum* with water and prepare the silver nitrate ($AgNO_3$) solution of different concentration viz. 1mM, 3mM and 5mM. After the preparation of flowers extract and silver nitrate, we mixed the flowers extract and silver nitrate in following ratios (Table 2).

On mixing the plant extract and $AgNO_3$ solution of different concentration, leave the solution for 4-5 days for reduction of Ag^+ to Ag. Then the reduced solution, centrifuged in Remi R8C Laboratory centrifuge machine at 5000 rpm. Separate the precipitate and dry in shade.

4 Characterization Techniques

After preparation of nanoparticles, it becomes essential to characterize them in terms of size, composition, phase, micro strain, etc. For the characterization of nanoparticles some laboratory techniques are essential. Following techniques have been used for characterization in the thesis.

i) Visual examination.
ii) Ultraviolet Visible (UV-Vis) Spectroscopy
iii) X- Ray Diffraction (XRD) by the
iv) Scanning Electron Microscopy (SEM)
v) Transmission Electron Microscopy (TEM)

All Ultraviolet Visible (UV-Vis) Spectroscopy were taken by Electronics India (EI)Double beam spectrophotometer model 3375, XRD measurements

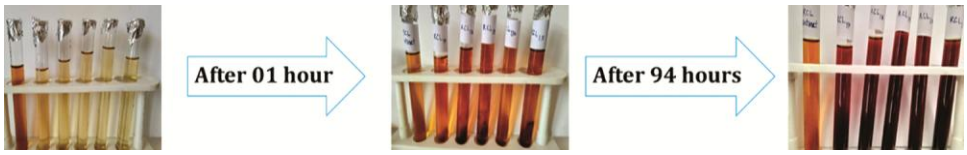


Fig. 2 — Visual examination[13] (Pictures captured by author).

of all the prepared samples were taken, at room temperature, by an X- ray diffractometer (PAN alytical, X'PERT PRO), using Cu Kα1 radiation of wavelength 1.5405980 Å radiation, in a wide range of 2θ (20°< 2θ < 70°) at a scanning rate of 6°/min. Surface morphology and grain size of the prepared samples were studied using a scanning electron microscope (CARL ZEISS, MA15/EVO18), and Transmission Electron Microscopy (TEM) were taken at HV 120kV.

5 Results and Discussion

5.1 Visual examination

The primary confirmation of the synthesized silver nanoparticles is done by visual basis. The color change of *R. campanulatum* flowers (RCF) extract and silver nitrate solution with respect to time was observed[12]. On the addition of *R. Campanulatum* leaves (RCL) extract to silver nitrate solution, entire mixture color changes from whitish yellow to brown red color indicates the formation of silver nanoparticles (Shown in Fig. 2). This formation indicates that silver ions in reaction medium have been converted to elemental silver having the size of nanometric range[13].

5.2 UV-Visible analysis

UV Visible spectral analysis characterizes the formation and completion of silver nanoparticles. Silver nanoparticles exhibit Plasmon absorption band in the visible region. The metal nanoparticles have free electrons which gives the surface Plasmon resonance absorption band due to the combined vibrations of electrons of metal nanoparticles in resonance with light wave. Silver nanoparticles are known to exhibit UV-Visible absorption in the range of 400-500 nm [13]. The absorption bands of silver nanoparticles were observed at 431nm for 1mM (RCL1:9), 473nm for 2mM(RCL1:11), 452nm for 3mM (RCL1:9),436 nm for leaves of *R. Campanulatum* and 464nm for 1mM (RCF1:9), 472nm for 5mM(RCF1:11) for flowers of *R. Campanulatum* and shown in Fig. 3.

5.3 XRD analysis

Figure 4 indicates the diffraction pattern of silver nanoparticles which shows peaks corresponding to

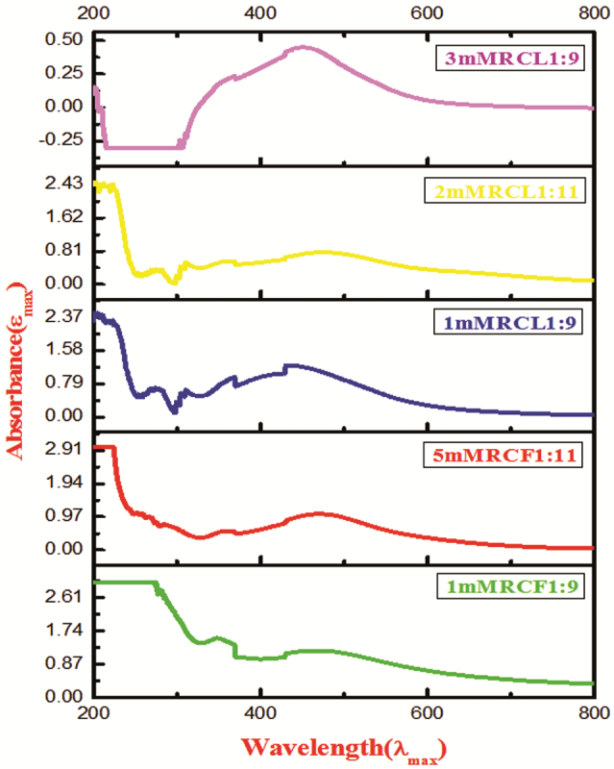


Fig. 3 — UV-Vis graph of AgNPs of *R.campanulatum.*

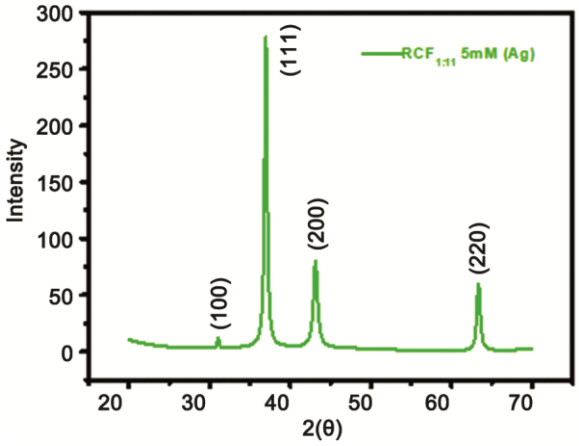


Fig. 4 — XRD graph.

elemental silver. XRD peak 2θ values observed at 37°,43° and 63°, which could be induced by the following crystalline planes ofsilver(111), (200) and (220), respectively. The obtained result reveals that

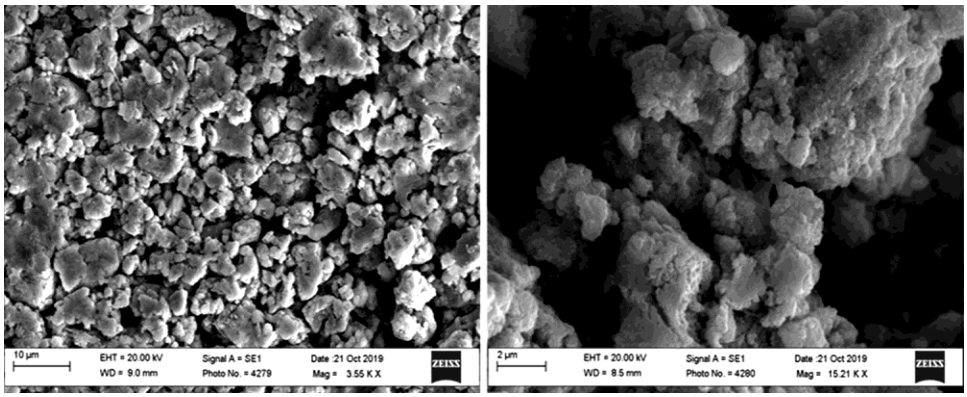
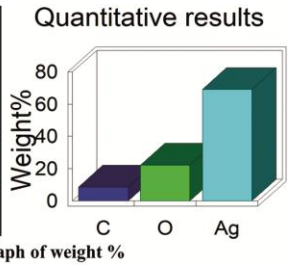


Fig. 5 — SEM Images.

Element	Weight %	Atomic %
C K	8.62	26.11
O K	22.24	50.58
Ag L	69.14	23.32



EDAX DATA &Graph of weight %

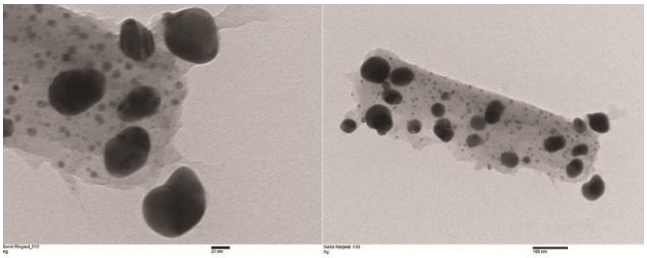


Fig. 6 — TEM Images.

the synthesized nanoparticle possess cubic crystalline phase. The lattice parameterfor this pattern was 'a' = 4.1280Å.

5.4 SEM and EDAX analysis

The SEM is especially useful for convenient inspection of grain structure. It produces 3D appearing Images of micro structural features and is a powerful tool for observing surface morphology. Surface morphology and grain size of prepared nanoparticles were studied using a Scanning Electron Microscope (CARL ZEISS MA15/EVO18). The prepared composition of $5RCF_{19}$ average grain size was found 3.975 μm, some grains exceeds more than 8 μm with spherical and smooth surfaces (Fig. 5).

In the Energy Dispersive X-ray analysis (EDAX) result of prepared sample $5RCF_{1:11}$ observed that the significant Ag was found with 69.14% of weight percentage in the synthesized nanoparticles.

5.5 TEM analysis

The morphology, structure and size of the synthesized nanoparticles were also examined through Transmission Electron Microscopy (TEM HV120kV). During TEM analysis, the samples were loaded on the copper grids andfurther dried under an IR lamp. The analysis of the loaded sample grids is performed at 120 kV. The average particle size was further calculated manually by taking the minimum and maximum particle size in the image (see Fig. 6).In the TEM analysis of given sample $RCF_{1:11}$ of 5mM average grain size has been observed 43.75nm, while some grains size exceeds more than 80nm.

6 Conclusions

The present research focused on the green synthesis of silver nanoparticles using *R. Campanulatum* flowers ($RCF_{1:11}$) extract. The flowers extract contains organic molecules which act as a bio-reduction and bio-stabilizing agent during the formation of silver nanoparticles. Furthermore, the silver nanoparticles formation is also confirmed by different nanoparticles characterization techniques. The UV–Vis observation shows the surface plasmonic resonance peak between 350 to 550 nm with a maximum absorption peak at 472nm for 5mM ($RCF_{1:11}$) for flowers of *R. campanulatum*, which confirms the formation of AgNPs. XRD confirms that the synthesized nanoparticle exhibits cubic crystalline structure.SEM image shows the agglomerated particles with spherical and irregular shapes. TEM analysis confirms the average grain sizes of silver nanoparticles were found to be 43.75nm with spherical morphology.

Acknowledgement

Authors are thankful to Mr. Prashant Thapliyal, USIC, HNB Garhwal University for SEM and EDX report of given samples.Also thankful to Dr. Surendra Singh, Deptt. of Physics (USIC), HNB Garhwal University for his valuable suggestions.

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